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INTERNATIONAL BULLETIN
OF PLANT PROTECTION

1945 Nos. 3 & 4

CHECKING OF ACCURACY IN TESTING FUNGICIDES 'IN VITRO', AND EXAMPLES PARTICULARLY AS APPLIED TO *ALTERNARIA, TENUIS* TYPE *

We have already spoken elsewhere of the sources of error which influence the results of laboratory testing of the fungicidal power of a mixture, with the method of destroying the germinating capacity of the conidia of *Alternaria, tenuis* type, previously described and discussed. The less standard and uniform the working conditions, the greater are the errors of evaluation which may occur with this method. These errors may also affect the results to the extent that they can no longer be taken into account.

But even if the conditions under which the tests are carried out can be completely standardized in all phases of the experiment (from preparation of the fungus to correction and graphic expression of the results), there still remain, however, extraneous sources of error : for example, the difficulty of diluting exactly the mixture and of rendering the dilution homogeneous at the time of spraying ; the errors due to the differences in germination deriving from intrinsic causes inherent in the fungus and its variability, etc.

It may be useful, therefore, to verify periodically the total accuracy of one's method of work, even for the sources of error most difficult to check, as they are inherent in the varying susceptibility of *Alternaria* to copper salts. This check up is also useful when apparently abnormal results are obtained with the germinations of the control fungus in water or in the selected standard fungicidal product (Bordeaux mixture, Caffaro powder).

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* Communication from Prof. R. CIFERRI, Director of the Botanical Institute of the University and the Italian Cryptogamic Laboratory, Pavia, Italy.

The test method which may be advantageously applied is that suggested by WILCOXON and MCCALLAN (1939) for the correction of extraneous sources of error by means of the chi-square test (X^2) †.

For each concentration of the fungicide examined, X^2 is calculated between the percentage of non-germinated spores (or germinations) effectively observed and that as read from the plotted line. The sum of the X^2 thus obtained is divided by the number of degrees of freedom n and the square root of this quotient is the correction factor to allow for extraneous sources of error :

$$\sqrt{S\ (X^2)\,/\,n}$$

The two afore-mentioned American investigators advise combining an appreciable number of these data (obtained in all laboratories with the same technique) in order to establish the average coefficient of correction.

* * *

We now take from our experiment reports a series of typical plottings obtained with the same fungicide and the same fungus, working under the same conditions but using different technical methods, which will elucidate the application of this checking up principle.

Fungicide : Caffaro powder with copper oxychloride containing 16 per cent. copper. Seven progressive dilutions (respectively 16, 8, 4, 2, 1, 0.5, 0.25 per cent. metal). Readings of 100 conidia on a drop on each slide. The other working conditions are the same as those described in our previous notes.

(1) Free hand spraying of slides : coefficient of error (C.E.) 2.79.

(2) Idem with the use of a wooden rule (according to BORZINI : see previous note) : C. E. 2.23.

(3) Idem with uniform pressure but without checking time of spraying : C. E. 2.01.

(4) Idem with control of duration of spraying but not of pressure : C. E. 1.69.

(5) Idem with control of duration and pressure of spraying on fixed slides : C. E. 1.47.

(6) Idem as in (5) but on slides placed on a revolving disc : C. E. 1.41.

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† The aforesaid factor for the correction of extraneous sources of error is derived from the quotient of deviation of LEXIS, in ratio to X^2 for the degrees of freedom :

$$Q.\ n = X^2 \text{ whence } Q = \frac{X^2}{n}$$

FISCHER (1934) notes that X^2, in reality, completes the LEXIS quotient, which is equivalent to the ratio between empiric standard deviation and standard deviation calculated for a BERNOULLI distribution ; if this ratio is less than one, there is an indication of 'supernormality of ratio'. It is consequently an index fully capable of expressing how many times the zone of error is greater than that which may be anticipated after a normal distribution. What is not so clear is why the two American investigators utilize the square root of this value.

From a simple examination of these figures it may at once be deduced that the most numerous causes of error derive from the duration of spraying and the pressure of spraying, more so for the former than for the latter ; the revolving disc makes for very little improvement in the accuracy of the experiment ; the simple wooden rule employed by BORZINI is already very advantageous compared with free hand spraying, etc.

* * *

A perhaps even more interesting application was effected in the study of error inherent — with all other factors equal — in the 'vitality' of the conidia of *Alternaria, tenuis* type, in the course of experiments carried out on different occasions under equal uniform and controlled conditions according to the technique which we previously described. We indicate below the most significant data:

Series (*A*) Age of cultures:—

5 days	C. E. 1.48
10 »	» 1.44
20 »	» 1.51
1 month	» 1.50
2 months	» 1.96
about 3 »	» 2.03
» 6 »	» 2.14

Series (*B*) Growth and purity of cultures:—

Normal cultures : C. E. 1.55.

Cultures which developed at a high temperature (about 30°C.) where specification is very weak and the colonies are formed chiefly of sterile aerial mycelium : C. E. 1.89. Cultures contaminated by a bacterium which was transported in the conidia suspension globules on the slides : C. E. 2.86.

Series (*C*) Density of conidia suspension in drops of water:—

Normal density	C. E. 1.52
Approximately double normal density	» 2.02
Four times normal density	» 3.47

Series (*D*) Germination temperature of conidia:—

Approximately 20-21°C.	C. E. 1.48
» 24-26°C.	» 1.49
» 28-30°C.	» 1.55
» 37°C.	» 2.22

Series (*E*) Time of incubation of conidia:—

Approximately	6 hours	C. E. 4.54
»	12 »	» 2.29
»	24 »	» 1.76
»	48 »	» 2.13
»	72 »	» 3.12

Discussion of results.

First of all it should be noted that the coefficients of error (C. E.) as calculated above have a relative value and cannot be taken as absolute values, that is to say that these series cannot be compared with each other since they were effected at different periods, which inevitably leads to unavoidable errors in each series (for example, the susceptibility of *Alternaria* to copper ; accuracy of dilutions and homogeneity of suspensions during spraying, etc.). On the other hand, the data of each series can be fully compared since the tests were carried out contemporaneously. According to the data given above, it could not be deduced that the germination temperature of the conidia of 37°C. (C. E. 2.22) relates approximately to the same C. E. (2.25) for the 12 hours incubation or that of the 6 months old culture (2.14).

The following are the fundamental conclusions derived from these conclusions : —

(1) In testing the fungicidal power of an 'in vitro' checked preparation with *Alternaria, tenuis* type, the conidia of colonies grown normally up to one month can be employed ; afterwards, the accuracy of the test begins to fall off, though as yet very little ;

(2) Cultures grown at a temperature appreciably higher than the optimum temperature, namely, at 30°C. instead of 20-21°C., are less favourable ;

(3) The inaccuracy of the test becomes appreciable when working with cultures contaminated with extraneous organisms (in our case, a bacterium) ;

(4) A suspension density of the conidia in the drop double normal prejudices the accuracy of the test which becomes still less exact with a quadruple concentration. There are possibly different causes to account for this : for example, an unequal concentration of the fungicide in the drop of conidia germination, owing to the relatively rapid and strong absorption of copper by the conidia ; but undoubtedly simple sources of error also come into play, as, for example, the difficulty in distinguishing the number of non-germinated from germinated conidia ;

(5) The optimum germination temperature of conidia in pendent drop lies between 20 and 26°C. ; as high as 30°C. it is not so good and up to 37°C. even less so ;

(6) The best time for reading the germinations of conidia germinated in the drops is after 24 hours ; 12 and 48 hours do not give such accurate results ; and even less so after 72 hours and only 6 hours. This may easily be explained by

noting that after 6 hours incubation germinations are not yet complete. After 72 hours the main error in the test is due probably to the fact that germinated tubes are long and ramified and inter-cross making it very difficult to distinguish the non-germinated from the germinated conidia.

Although like results could be attained with the calculation of X^2 according to the usual method employed in biometry, the use of the coefficient of error as per WILCOXON and MCCALLAN is more convenient in that it renders ' visual ', so to speak, the accuracy of the experiment technique, and makes it possible to follow and control improvement and also to estimate the ' value ' of each technical method employed in effecting the test, provided, naturally, that the ' in vitro ' testing of fungicides is carried out under uniform and stable conditions.

* * *

According to our experience, the following generic calculations of the accuracy of the check method correspond to the different coefficients : —

C. E. 1.4-1.7. Method of operating exactly standardized ; accurate control of all stages of execution. This is the average coefficient of error when a maximum checking technique is adopted.

C. E. 1.7-2. Method of operating only partially standardized ; most, but not all the stages of execution checked. This accuracy (and the relative C. E.) may be attained if equipment is limited though having available a very careful and expert staff *.

C. E. 2-2.3. Method of operating standardized only to a small extent ; only the smallest part of the stages of execution checked. This degree of accuracy is obtained with makeshift equipment and inadequately trained staff.

C. E. higher than 2.3. Non-standardized method of operation, practically no control of the different stages of execution. Little or no account can be taken of the results.

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* WILCOXON and MCCALLAN, before having improved to the utmost the method described in the latest reports, calculated their mean coefficient of error at about 2.

THIRD CONTRIBUTION TO THE LOCUST BIBLIOGRAPHY *

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* Drawn up by GIULIO TRINCHIERI, International Institute of Agriculture.

† See this *Bulletin* 1944, Nos. 3-4, pp. 28-32 ; Nos. 5-6, pp. 39-48 ; Nos. 7-8, pp. 54-64 ; Nos. 9-10, pp. 72-80 ; Nos. 11-12, pp. 92-100.

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